

Effect of high temperature on the metabolic processes affecting sorbitol synthesis in the silverleaf whitefly, *Bemisia argentifolii*

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Abstract

Whiteflies accumulate the polyhydric alcohol, sorbitol, when exposed to temperatures greater than about 30°C. Feeding experiments using artificial diets containing labeled sucrose showed that more of the label was incorporated into whitefly bodies and less was excreted in the honeydew when feeding was conducted at 41 compared with 25°C. Analysis of the components of the honeydew showed that more of the excreted label was in glucose and fructose and less in trehalulose at 41°C than at 25°C. A similar effect of temperature on honeydew composition occurred for whiteflies feeding on cotton leaves. Measurement of the activities of glycolytic, pentose-phosphate and polyol pathway enzymes at 30 and 42°C showed that NADPH-dependent ketose reductase/sorbitol dehydrogenase (NADPH-KR/SDH), sucrose, glucokinase and glucose-6-phosphate dehydrogenase activities were stimulated to a greater extent at 42°C than trehalulose synthase and fructokinase. NAD⁺-sorbitol dehydrogenase (NAD⁺-SDH) activity was inhibited at 42°C. We propose that high temperature alters metabolic activity in a way that increases the availability of fructose and stimulates pentose-phosphate pathway activity, providing both the substrate and coenzyme for sorbitol synthesis. High temperature also increases the activity of NADPH-KR/SDH, the enzyme in whiteflies that synthesizes sorbitol, but inhibits the activity of NAD⁺-SDH, the enzyme that degrades sorbitol. Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

Silverleaf whiteflies, *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae), thrive in desert regions where they can attain almost plague-like populations on some crop species (Gerling et al., 1980; Byrne et al., 1990; Henneberry et al., 1995). Air temperatures in this environment can reach 50°C, presenting a major challenge to insect survival. Many insects cope with high environmental temperatures by using evaporative water loss to cool their body temperature (May, 1985; Prange, 1996). However, small insects like the silverleaf whitefly have a large surface area to volume ratio that precludes evaporative cooling as a mechanism for thermoregulation (Prange, 1996). For these insects, desiccation is another major environmental stress, which for whiteflies and other phloem-feeding insects is further complicated

by the high osmotic strength of a phloem sap diet (Downing, 1978; Fisher et al., 1984; Wilkinson et al., 1997).

Previously, we showed that the polyhydric alcohol, sorbitol, accumulates in whiteflies to hemolymph levels in excess of 0.4 M when these insects are exposed to temperatures greater than about 30°C (Wolfe et al., 1998). Polyols stabilize the native conformation of proteins, counteracting the detrimental effects of desiccation and temperature extremes. Numerous insect species accumulate polyols as colligative cryoprotectants to increase cold-hardiness (reviewed in Denlinger et al., 1991; Lee, 1991; Storey and Storey, 1992). Our studies with whiteflies (Wolfe et al., 1998) and aphids (Hendrix and Salvucci, 1998) suggest that polyols also function as thermoprotectants that protect these insects against the detrimental effects of high temperature. This conclusion is consistent with the well-known ability of polyols to protect enzymes against thermal denaturation (Back et al., 1979; Kim and Lee, 1993; Erarslan, 1995; Wimmer et al., 1997).

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The enzyme catalyzing sorbitol synthesis in whiteflies is an unusual ketose reductase that uses NADPH to reduce fructose to sorbitol (Wolfe et al., 1998; Salvucci et al., 1998). Whiteflies also contain a NAD⁺-dependent sorbitol dehydrogenase (NAD⁺-SDH), the normally catabolic enzyme that oxidizes sorbitol to fructose (Wolfe et al., 1998). Previously, we showed that dietarily-derived fructose was diverted to sorbitol synthesis when ambient temperatures were increased from 28 to 41°C (Wolfe et al., 1998). In the present study, we determine the mechanism for increased accumulation of sorbitol at high temperature by examining the effect of temperature on the fate of ingested carbon and the activities of sorbitol, glycolytic and pentose-phosphate pathway enzymes.

2. Materials and methods

2.1. Chemicals

The radiolabeled sugar, [U-¹⁴C]sucrose, was purchased from Amersham Life Science (Arlington Heights, IL)¹. Unless indicated otherwise, biochemical reagents were from Sigma Chemical Co. (St. Louis, MO).

2.2. Insect and plant material

Adult silverleaf whiteflies were reared within a glasshouse on cotton (*Gossypium hirsutum* L., cv. Coker 100A glandless) as previously described (Salvucci et al., 1997). The temperature in the glasshouse varied diurnally from 25°C in the morning to 38°C by 2:00 pm. Whiteflies were collected from colony plants by gentle aspiration.

2.3. Feeding experiments

For radiolabeling studies, whiteflies were supplied with artificial diets containing 20% sucrose and trace amounts of [¹⁴C]sucrose (Salvucci et al., 1997). After 4 h of feeding at 25 or 41°C, whiteflies from two to four separate feeders were collected and the amount of ¹⁴C in the bodies and in the excreted honeydew was determined by liquid scintillation spectrometry. Components in the honeydew and 80% ethanol-soluble fraction of the body were separated by HPLC and the amount of ¹⁴C in the various compounds was determined by liquid scintillation spectrometry (Wolfe et al., 1998). The data presented are the means of at least two separate experiments.

For honeydew collection, approximately 100 white-

flies were placed in clip cages on the under surface of intact cotton leaves. After 1 h in the light (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) at 25°C, clip cages were removed and the plants were enclosed in water-jacketed beakers as described previously (Wolfe et al., 1998). The temperature inside the beakers, monitored with a thermocouple, was either maintained at 25°C or increased slowly over a 2 h period from 25 to 40°C. Honeydew was collected at 1 h intervals by placing plastic dishes under the leaves. Honeydew was recovered from the dishes by dissolving the droplets in water.

2.4. Extraction and analysis of carbohydrates

Frozen whiteflies were extracted and their body contents were analyzed by anion-exchange HPLC using pulsed amperometric detection as described previously (Hendrix and Wei, 1994; Wolfe et al., 1998). Honeydew was also analyzed by HPLC after concentrating the dissolved material by lyophilization. The amounts of the various sugars and polyols were determined by comparing the peak areas with known amounts of standards.

2.5. Enzyme assays

Soluble whitefly extracts were prepared at 4°C by homogenizing approximately 200 glasshouse-reared adult whiteflies in 50 mM HEPES-KOH, pH 7.9. The extract was centrifuged for 10 min at 10,000g and the supernatant was used for determining the activities of the various enzymes. Assays were conducted at the temperatures indicated in the text. Soluble protein in the extracts was determined by a dye-binding assay (Bradford, 1976). Sucrase (α -glucopyranosidase) and trehalulose synthase activities were determined with saturating sucrose concentrations after extraction in 1% (v/v) Triton X-100 (Salvucci et al., 1997). NADPH-KR/SDH and NAD⁺-SDH activities were measured in the direction of sorbitol formation using saturating concentrations of fructose as described previously (Salvucci et al., 1998). Fructo- and glucokinase activities were determined at saturating substrate concentrations by monitoring the glucose- or fructose-dependent increase in absorption at 340 nm in an assay system linked to the reduction of NADP⁺ via Glc-6-P dehydrogenase. Assay mixtures contained 80 mM MOPS-KOH, pH 7, 8 mM MgCl₂, 2 mM ATP, 2 mM NADP⁺, 2 IU phosphoglucosomerase, 1 IU Glc-6-P dehydrogenase, 40 mM fructose or glucose and whitefly extract. Phosphoglucosomerase activity was measured using a similar reaction, but lacking commercial phosphoglucosomerase and containing 20 mM Fru-6-P instead of glucose or fructose. Glucose-6-phosphate dehydrogenase activity was determined by measuring the Glc-6-P-dependent increase in absorption at 340 nm caused by NADP⁺ reduction. Reaction mixtures contained 80 mM MOPS-KOH, pH 7, 8 mM

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MgCl₂, 2 mM NADP⁺, 20 mM Glc-6-P and whitefly extract. Phosphofructokinase activity was determined by measuring the Fru-6-P-dependent rate of NADH oxidation in a reaction mixture containing 80 mM MOPS-KOH, pH 7, 8 mM MgCl₂, 2 mM ATP, 0.3 mM NADH, 10 mM Fru-6-P, 5 IU aldolase, 5.4 IU triose-P isomerase, 7.7 IU glycerol-P dehydrogenase and whitefly extract. All assays were conducted in triplicate and the results presented are the means \pm SEM.

3. Results

3.1. Effect of temperature on the ingestion and metabolism of [¹⁴C]sucrose

The amount of ¹⁴C incorporated and excreted at 25 and 41°C was determined for whiteflies that had fed for 4 h on artificial diets containing [¹⁴C]sucrose (Table 1). The total amount of label recovered in the bodies was similar at the two temperatures, but less of the incorporated label was excreted in the honeydew at 41°C compared with 25°C. In contrast, the amount of ¹⁴C recovered in the bodies of the whiteflies was greater when feeding was conducted at 41°C compared with 25°C. On a percentage basis, 76 and 62% of the recovered label was in the whitefly bodies at 41°C and 25°C, respectively. Most of the label in the bodies of the whiteflies was soluble in 80% ethanol.

The distribution of ¹⁴C among the various sugars and polyols in the honeydew and in the bodies of the whiteflies was determined by anion-exchange HPLC (Fig. 1). At 25°C, label from ingested sucrose was primarily associated with trehalose, glucose and an unknown carbohydrate in the body of the insect (see also Wolfe et al., 1998). These compounds were also labeled at 41°C, but the majority of the label at 41°C was associated with sorbitol. For whiteflies feeding at 25°C, most of the label in the honeydew was in the form of trehalulose (Fig. 2). Trehalulose was also the major labeled sugar in the honeydew from whiteflies feeding at 41°C, however, a

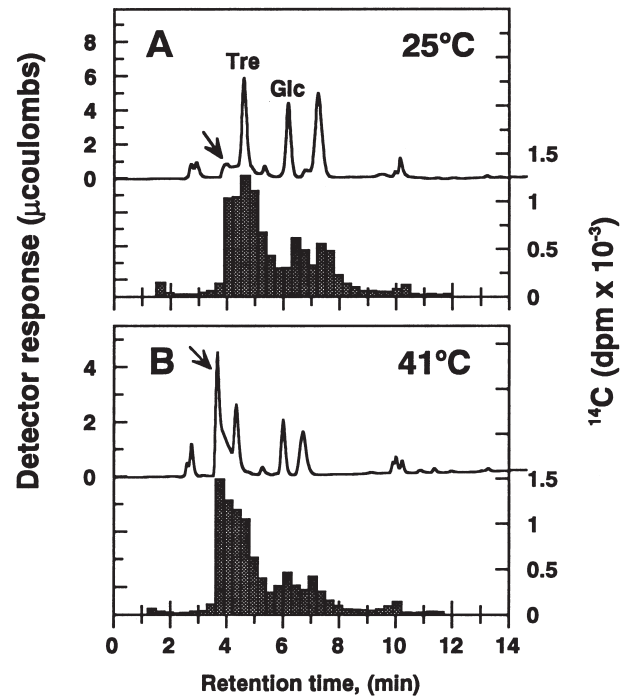


Fig. 1. Distribution of ¹⁴C among components of the whitefly after feeding on [U-¹⁴C]sucrose at 25 and 41°C. Whiteflies were supplied with artificial diets containing 20% sucrose and trace amounts of [U-¹⁴C]sucrose. After 4 h at 25 (A) or 41°C (B), whiteflies on four separate feeders, each containing 20 individuals, were collected. Components of the 80% ethanol-soluble fraction of whitefly bodies were separated by anion-exchange HPLC and detected by pulsed amperometry (lines). The amount of ¹⁴C in the various compounds (bars) was determined by liquid scintillation spectrometry. Arrows indicate the position of sorbitol in the chromatograms. Tre = trehalose; Glc = glucose.

significant proportion of the label was also associated with glucose and fructose.

That a greater percentage of ¹⁴C label in the honeydew was in glucose and fructose at 41 compared with 25°C suggested that the ratio of sucrose hydrolysis/isomerization was greater at the higher temperature. To determine if a change in the monosaccharide/disaccharide (i.e. glucose + fructose/trehalulose) ratio

Table 1

Distribution of ¹⁴C in whiteflies after feeding at 25 and 41°C on artificial diets containing [U-¹⁴C]sucrose

Component	Amount incorporated (nmol C whitefly ⁻¹)*		Percent incorporated (%)	
	25°C	41°C	25°C	41°C
honeydew	17.4 \pm 3.4	10.9 \pm 0.8	38	24
body				
soluble	24.4 \pm 0.9	32.7 \pm 2.7	53	72
insoluble	4.2 \pm 1.1	2.6 \pm 1.6	9	4
total	46	45.2		

*Nanomoles ¹⁴C per whitefly after feeding for 4 h. Results are the mean values \pm SEM of two separate experiments at each temperature each using two feeders with 20–30 whiteflies per feeder.

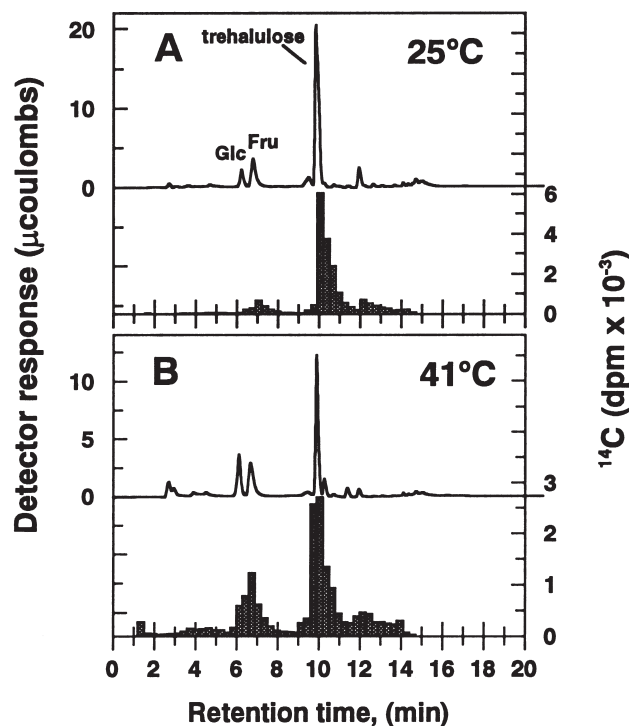


Fig. 2. Distribution of ^{14}C among components of the whitefly honeydew after feeding on $[\text{U-}^{14}\text{C}]$ sucrose at 25 and 41°C. Whiteflies were supplied with artificial diets containing 20% sucrose and trace amounts of $[\text{U-}^{14}\text{C}]$ sucrose. After 4 h at 25 (A) or 41°C (B), the honeydew from whiteflies on four separate feeders, each containing 20 individuals, was collected. Components of the whitefly honeydew were separated by anion-exchange HPLC and detected by pulsed amperometry (lines). The amount of ^{14}C in the various compounds (bars) was determined by liquid scintillation spectrometry. Glc = glucose; Fru = fructose.

also occurs during plant feeding, we examined the effect of temperature on the composition of the honeydew from whiteflies feeding on cotton leaves. At 25°C, the ratio of glucose and fructose to trehalulose was 0.33 (Fig. 3). As temperature increased above 25°C, this ratio increased markedly. After 1 h at 40°C, the ratio of glucose plus fructose to trehalulose in the honeydew of whiteflies feeding on cotton was 1.5, after 2 h at 40°C the ratio was 2.5.

The activities of the enzymes that isomerize and hydrolyze sucrose were measured at several different temperatures (Fig. 4). Over the range of temperatures from 35 to 50°C, sucrase activity increased with temperature whereas trehalulose synthase activity was either unchanged or decreased from its optimum at 35°C. Compared to its maximal activity, trehalulose synthase activity was inhibited by 32.5% at 55°C. In contrast, sucrase activity was inhibited by only 15% at this temperature.

The activities of several glycolytic, pentose-phosphate, and sorbitol pathway enzymes were determined at 30 and 42°C in soluble extracts prepared from whiteflies (Table 2). These two temperatures were used because whiteflies accumulate much higher levels of sorbitol at

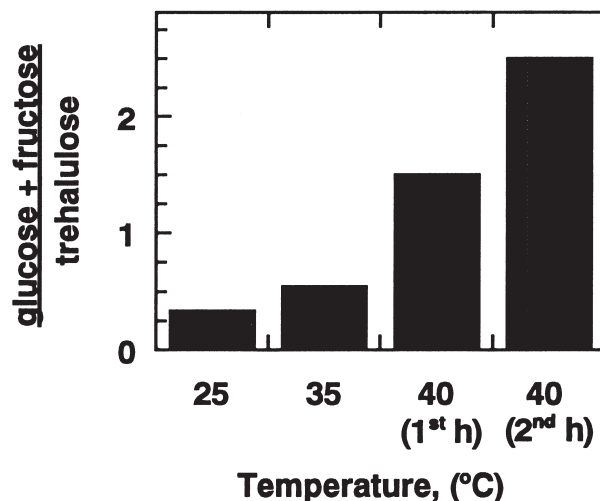


Fig. 3. The effect of temperature on the composition of whitefly honeydew. Whiteflies (approximately 100 adults) were fed in the light on cotton plants at 25°C, before increasing the temperature to 35°C for 1 h, and then to 40°C for 2 h. Honeydew was collected every 1 h and then analyzed for carbohydrates.

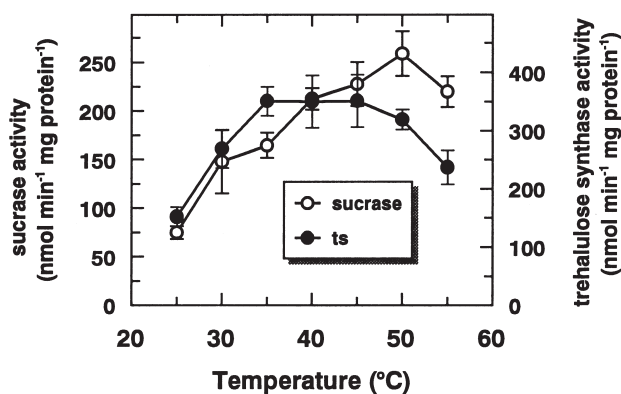


Fig. 4. The effect of assay temperature on the activities of trehalulose synthase and sucrase in whitefly extracts.

42°C than at 30°C. Glucose-6-P dehydrogenase and glucokinase showed the greatest change in activity between 30 and 42°C, increasing 2.2- and 2-fold, respectively. In contrast, there was little difference in the activity of fructokinase between the two temperatures. The activities of two glycolytic enzymes, phosphofructokinase and phosphoglucosyltransferase were 1.7- and 1.5-fold higher, respectively, at 42 compared with 30°C. The activity of the sorbitol biosynthetic enzyme, NADPH-KR/SDH, was 1.6-fold higher when assayed at 42 compared with 30°C. In contrast, the activity of the sorbitol degradative enzyme, NAD⁺-SDH, was less at 42 than at 30°C.

4. Discussion

Whiteflies metabolize ingested sucrose by either hydrolyzing it to its monosaccharide units, isomerizing it

Table 2

Effect of assay temperature on the activities of glycolytic, pentose-phosphate and polyol pathway enzymes in whiteflies

Enzyme	Activity ($\mu\text{mol min}^{-1} \text{ m protein}$)		Ratio $\text{act}_{42}/\text{act}_{30}^a$
	30°C	42°C	
trehalulose synthase	0.54 ± 0.05	0.77 ± 0.04	1.4
sucrase	0.43 ± 0.05	0.72 ± 0.04	1.7
fructokinase	0.43 ± 0.03	0.46 ± 0.01	1.1
glucokinase	0.53 ± 0.01	1.06 ± 0.06	2.0
phosphoglucosomerase	1.9 ± 0.09	2.92 ± 0.14	1.5
Glc-6-P dehydrogenase	0.14 ± 0.01	0.31 ± 0	2.2
phosphofructokinase	0.34 ± 0.05	0.58 ± 0.07	1.7
NADPH-KR/SDH	0.77 ± 0.05	1.25 ± 0.08	1.6
NAD ⁺ -SDH	0.11 ± 0	$< 0.11^b$	< 1

^aRatio of the activity at 42°C to the activity at 30°C; ^bEnzyme activity decreased continually during the assay from $0.11 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$ to near zero.

to trehalulose (α -D-glucose (1,1) D-fructose), or converting it to longer chain oligosaccharides (Hendrix and Wei, 1994; Salvucci et al., 1997). Trehalulose and the longer chain oligosaccharides are excreted in the honeydew, whereas glucose and fructose are either excreted or incorporated into the body for use in metabolic reactions. Previously, we showed that whiteflies feeding at 41°C accumulated labeled sorbitol when labeled fructose was supplied in the diet, but not when the source of the label was glucose (Wolfe et al., 1998). This result indicates that one of the metabolic uses of dietarily-derived fructose at elevated temperatures is for the synthesis of sorbitol.

In the present study, exposure of whiteflies to temperatures $\geq 35^\circ\text{C}$ increased the ratio of glucose and fructose to trehalulose in the honeydew. This change in honeydew composition occurred on both artificial and plant diets and was consistent with the effect of temperature on the enzymes that hydrolyze and isomerize sucrose. Thus, high temperatures increased the amount of fructose and glucose available for uptake by stimulating sucrose hydrolysis at the expense of sucrose isomerization. Our data showed that the additional hexose available at higher temperatures is metabolized by the whiteflies accumulating as sorbitol, probably in the hemolymph (Table 1 and Fig. 1).

The K_M of whitefly NADPH-KR/SDH for fructose is about 600 mM (Salvucci et al., 1998), similar to the concentration of sucrose in plant phloem sap (Fisher and Gifford, 1986). Because of the low affinity of this enzyme for fructose, higher concentrations of fructose at higher temperatures would be expected to increase the rate of sorbitol synthesis. That whiteflies incorporated more of the ingested carbon in their bodies at 41 compared with 25°C indicates that hexose transport is also faster at the higher temperature. The faster rate of hexose transport may be the result of a direct effect of temperature on the activity of the hexose transporter protein. Alternatively, higher concentrations of hexoses at the

higher temperatures may increase the flux through the hexose transporter.

The pentose-phosphate pathway appears to play a major role in regulating polyol synthesis in insects that accumulate polyols for cold-adaptation (Storey and Storey, 1992; Holden and Storey, 1994). This pathway provides NADPH, the coenzyme required for the reduction of glucose to sorbitol or glyceraldehyde to glycerol (Yamashita et al., 1975; Wood and Nordin, 1980; Storey and Storey, 1992). Using differentially labeled glucose, Wood and Nordin (1980) showed that pentose-phosphate pathway activity increases relative to TCA pathway activity in *Protophormia terranova*, an insect species that accumulates glycerol in response to cold, but not in *Musca domestica*, an insect species that does not accumulate glycerol. Similarly, Moreau et al. (1977) showed that colder temperatures increase the rate of pentose-phosphate pathway activity relative to glycolysis in *Bombyx mori* and *Pieris brassicae*. Thus far, no direct measurements have been made of pentose-phosphate pathway activity in whiteflies. However, the rapid synthesis and high accumulation of sorbitol at elevated temperatures indicate that ample NADP(H) must be available in the reduced form to drive the increased rate of polyol synthesis. Also, measurements of enzyme activities at 30 and 42°C showed that the activity of Glc-6-P dehydrogenase, the key enzyme of the pentose-phosphate pathway, increased with temperature more than all of the other enzymes measured, including the glycolytic enzyme, phosphofructokinase.

4.1. Metabolic regulation of sorbitol synthesis

Measurements of sorbitol accumulation, honeydew composition, and the activities of glycolytic, pentose-phosphate and sorbitol pathway enzymes, suggest a metabolic sequence to account for the accumulation of sorbitol in whiteflies at elevated temperatures (Fig. 5). First, elevated temperatures increase the availability of

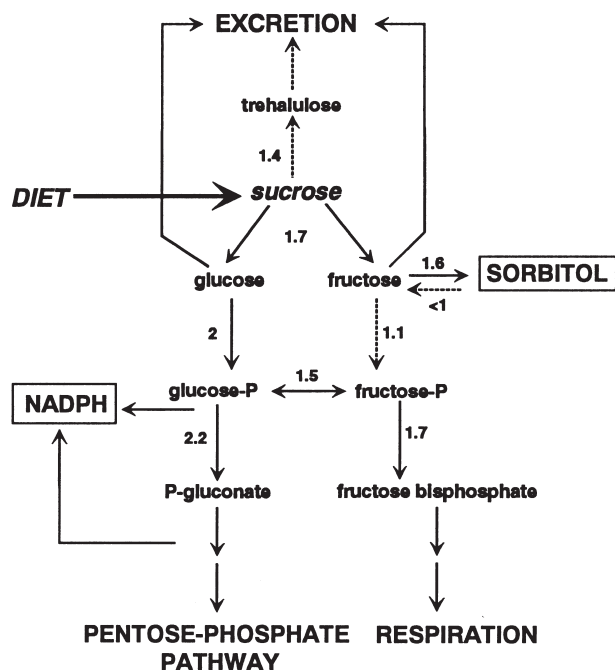


Fig. 5. The sequence of metabolic events leading to accumulation of sorbitol in whiteflies at elevated temperatures. The solid lines indicate the predicted flux of metabolites at elevated temperatures based on the effects of temperature on the activities (Table 2) and substrates of the various enzymes. The numbers indicate the -fold enhancement of activity between 30 and 42°C (Table 2). The broken lines indicate reactions that are catalyzed by enzymes whose activities were not enhanced at least 1.5-fold between 30 and 42°C.

fructose, the immediate precursor of sorbitol, by increasing the rate of sucrose hydrolysis to a greater extent than the rate of sucrose isomerization. Some of the free fructose liberated by hydrolysis is undoubtedly phosphorylated by fructokinase. However, the amount of fructose that is diverted through glycolysis may not change at elevated temperatures since fructokinase activity is not stimulated at elevated temperatures. Thus, the availability of free fructose would increase at elevated temperatures, which should increase the rate of sorbitol synthesis by overcoming the substrate limitation of NADPH-KR/SDH (Salvucci et al., 1998).

A second metabolic consequence of elevated temperatures appears to be an increase in flux through the pentose-phosphate pathway. Increased hydrolysis of sucrose at elevated temperatures would increase the amount of glucose available for phosphorylation. The effect of temperature on glucokinase and glucose-6-phosphate dehydrogenase activities suggests that the flux of glucose through the pentose-phosphate pathway increases at elevated temperatures. Increased flux through the pentose-phosphate pathway would provide a source of reduced pyridine nucleotide to support an increased rate of sorbitol synthesis. Finally, our results showed that NAD⁺-SDH activity was inhibited at 42°C, whereas NADPH-KR/SDH activity was highest at temperatures

between 40 and 55°C (Table 2 and Salvucci et al., 1998). Inhibition of NAD⁺-SDH activity at high temperature would contribute to the accumulation of sorbitol in the whitefly by preventing catabolism of sorbitol to fructose under conditions that favor sorbitol synthesis.

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